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## From platelet dust to gold dust: physiological importance and detection of platelet microvesicles

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This series of contemporary reviews focuses on the unique physiology and detection of platelet derived microvesicles. Platelet associated procoagulant activity, or “platelet dust”, has long been known to be important in haemostasis<sup>1,2</sup>. Defects in vesicle associated procoagulant activity are indeed implicated with various bleeding disorders<sup>3,4</sup>. Conversely, high levels of circulating platelet microvesicles are not only platelet specific activation markers, but are potential biomarkers of a variety of thrombotic<sup>5-7</sup>, inflammatory diseases<sup>8-10</sup>, and cancer<sup>11,12</sup>. Thus, profound understanding together with accurate and reproducible detection of platelet microvesicles may turn platelet dust into gold dust.

Fig. 1 shows a transmission electron micrograph of platelet microvesicles labelled with gold nanoparticles targeting integrin beta-3 (CD61). This single image reveals the presence, functionality, heterogeneity, as well as the small size of platelet microvesicles. Because platelet microvesicles are more than 10-fold smaller in diameter than platelets themselves, platelet microvesicles are difficult to study.

For many years, haemostasis and thrombosis researchers have measured platelet procoagulant vesicle activity using relatively simple clotting assays<sup>2,13,14</sup>. Although these assays are useful for measuring bulk procoagulant activity of vesicles, they provide little information on the individual properties of vesicles. Because vesicles are heterogeneous, access to the individual properties of vesicles is likely to provide more accurate clinical information.

Flow cytometry was the instrumentation of choice to characterize platelet microvesicles one by one<sup>3,4,9-14</sup>. The development of flow cytometry not only revolutionized the phenotypic and functional measurement of platelets, but also facilitated deeper insight into the size range, phenotypes and functional characteristics of platelet vesicles in whole blood, platelet-rich plasma (PRP) or platelet-poor plasma (PPP). However, flow cytometers were originally designed to measure cells and platelets, thus soon it became clear that there are fundamental physical limitations to the application of flow cytometers to characterize microvesicles<sup>15,16</sup>. The arrival of new biophysical approaches taught us that conventional flow cytometry is unable to characterise the full size range and potential enormous quantities of vesicles<sup>17</sup>. Despite this, an evolving series of studies led by the Scientific and Standardization Committee (SSC) on vascular biology of the International Society of Thrombosis and Haemostasis (ISTH), the International Society on Extracellular Vesicles (ISEV), and the International Society for Advancement of Cytometry (ISAC) have led to an optimal understanding of the size limitation of individual instruments<sup>18-21</sup>. Since one year, the joint flow cytometry working group of the ISTH, ISEV and ISAC provides important recommendations on pre-analytical and analytical variables to facilitate standardization of vesicle measurements ([www.evflowcytometry.org](http://www.evflowcytometry.org))<sup>22</sup>.

Meanwhile, pioneering engineers pushed the lower limit of size detection downwards<sup>21,23</sup>. Using these next generation cytometers, researchers showed that there was previously unknown biological information within the smaller vesicle populations. This tendency is now culminating in the development of dedicated nanocytometers<sup>24</sup>, designed from first principles to detect all vesicle populations. Coupled with the recent advancements in the field of extracellular vesicle research, it has become apparent that vesicles are not just potential biomarkers and important in many areas of cell biology, but provide a novel intercellular transport mechanism between different cell types.

This commissioned series of up to date reviews begins with two articles on the role of platelet microvesicles in health and disease and their novel roles in intercellular communication by Boilard and Edelstein, respectively<sup>25,26</sup>. Nieuwland and Gasecka then discuss whether platelet vesicles have utility as useful biomarkers of arterial thrombosis<sup>27</sup>. The remaining reviews focus on vesicle detection, including classical functional and genetic assays by Amiral et al.<sup>28</sup>; the detection of vesicles by immuno-based bulk techniques by Coumans et al.<sup>29</sup>, the detection of single vesicles in suspension using a variety of novel approaches by Buzas, Gardiner, Lee and Smith<sup>30</sup>; and the recent exciting developments in flow cytometry and nanocytometry by Nolan and Jones<sup>31</sup>. This series culminates with a new research paper by Brisson et al.<sup>32</sup>, demonstrating the beautiful structure of vesicles from activated platelets by cryo-electron microscopy.

In sum, the contributions to this review series justify that the field has progressed enormously in the last 20 years. It is evident that we are at the threshold of capitalizing on the enormous potential utility of vesicle measurements as biomarkers and as novel therapeutic agents for many of the major diseases that afflict mankind.

## Figure legend

Figure 1: Transmission electron microscopy (TEM) image of platelet microvesicles labelled with gold nanoparticles targeting integrin beta-3 (CD61). CD61 is a cluster of differentiation found on platelets. Microvesicles imaged by TEM often have a cup-shaped morphology, which is attributed to the thin (~5 nm) and thus fragile phospholipid membrane, which often collapses due to adhesion and dehydration. In suspension, however, most (>95%) microvesicles are spherical particles<sup>32,33</sup>. Other particles in this image may be proteins, lipoprotein particles or vesicles from other cell types than platelets.

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